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Human color cancer, WiDr cells suspended in MEM media containing 10% of fetal calf serum were seeded in 96-well microtiter plates at 2×10^3 cells/well, and precultured in an incubator with 5% of CO₂ at 37°C for 24 hours. Then, each compound diluted appropriately with the media was added into the well at 50 µl/well. At this time, a final concentration of each compound is up to 100 µmol/l or 1 µmol/l. The cells were cultured in the incubator with 5% of CO₂ at 37° for additional 72 hours. At 5 hours before the culturing was terminated, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide) dissolved in the medium at a final concentration of 1mg/mL was added into the wells at 50 µl/well. After the culturing, dimethyl sulfoxide was added to the well at 150 µl/well, and the plate was vigorously stirred using a plate mixer dissolve crystals of MTT-formazan completely. Then, the difference between absorbance at 550 nm and that at 630 nm was measured by a microplate reader. The antiproliferative activity for cellular proliferation was expressed as the concentration at which 50% inhibition of proliferation (IC₅₀) was induced. The results are shown in Table 5.

REMARKS

The specification has been amended to correct typographical errors. No new matter has been added.

Entry hereof is earnestly solicited.